

# Antigen-binding units

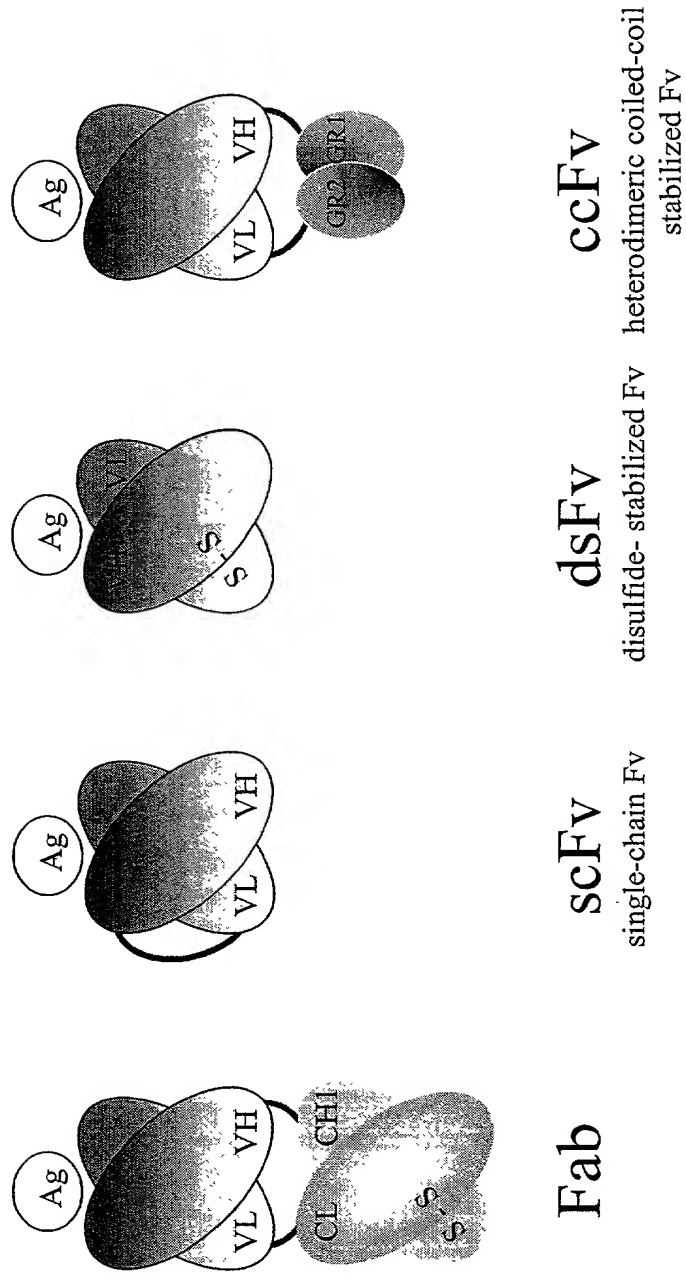


Fig. 1

# Sequences of coiled-coil domain in ccFv

GR1 Sequence Range: 1 to 146

XbaI	10	20	30	40	50											
	<u>TCTAGAGGTGGAGGAGTGAGAGAAATCCCGCTGTTGGAGAAGAGAA</u>															
	S	R	G	G	G	E	E	K	S	R	L	L	E	K	E	N
	60	70	80	90	100											
	<u>CCGTGAACCTGGAAGATCATCTGTGAGAAAGAGGCGTGTCTCTGAAC</u>															
	R	E	L	E	K	I	I	A	E	K	E	E	R	V	S	E
	110	120	130	140	AscI											
	<u>TGCGCCATCAACTCCAGTCTGTAGGAGTTGTTAATAGGCGCGCC</u>															
	L	R	H	Q	L	Q	S	V	G	G	C	*	*			

GR2 Sequence Range: 1 to 140

XhoI	10	20	30	40	50											
	<u>TCTCGAGGAGGTGGTGAACATCCCGCTGGAGGCGCTACAGTCAGAAAA</u>															
	S	R	G	G	G	T	S	R	L	E	G	L	Q	S	E	N
	60	70	80	90	100											
	<u>CCATCGCCTGCGAATGAAGATCACAGAGCTGGATAAAGACTTGGAAGAGG</u>															
	H	R	L	R	M	K	I	T	E	L	D	K	D	L	E	E
	110	120	130	NotI	140											
	<u>TCACCATGCAGCTGCAGGACGTGGAGGTTGCGGCGCGC</u>															
	V	T	M	Q	L	Q	D	V	G	G	C	A	A	A		

Fig. 2

# Vectors for antibody expression

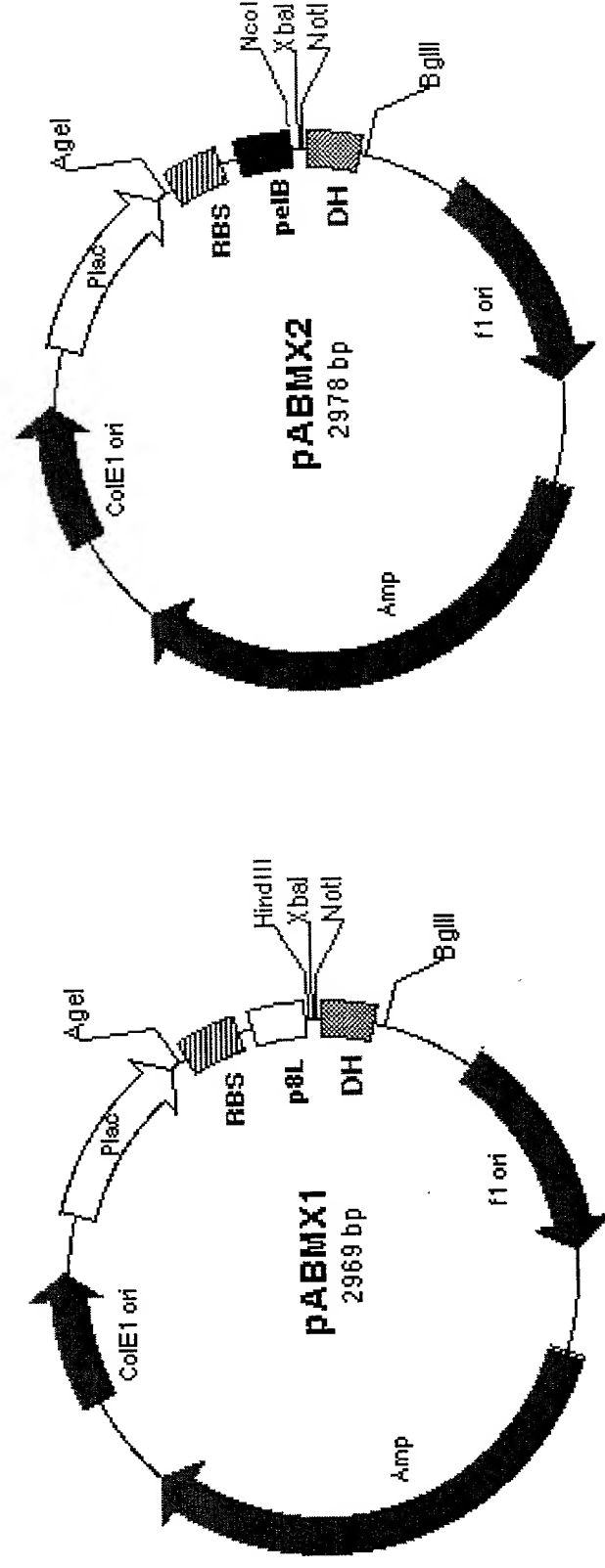


Fig. 3A

**PABMX1 vector: sequence from AgeI to BglII**

```

lac promoter/lac O1      AgeI      EP      S/D
AATTGTGAGCGGATAACAATT ACCGGT TCCT TTAACCTTAG TAAGGAGG AATTAAAAA
P8 Leader
ATGAAAAAGTCTTAGTCCTCAAAAGCCTCCGTAGCCGTTGCTACCCCTCGTTCCGATGCTAAGCTTCGCT TCTAGA XbaI
M K K S L V L K A S V A V A T L V P M L S F A S R
NotI
GCGGCCGCT TATCCATACGACGTACCAGACTACGCA GGAGGT CATCACCATCATCACCAT TAG AGATCT BglII
A A A Y P Y D V P D Y A G G H H H H H * R S

```

**PABMX2 vector: sequence from AgeI to BglII**

```

lac promoter/lac O1      AgeI      EP      S/D
AATTGTGAGCGGATAACAATT ACCGGT TCCT TTAACCTTAG TAAGGAGG AATTAAAAA
pelB Leader
ATGAAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCGGCCCGCCAGCCCGCCCTGCAGGCCCTCTAGA XbaI
M K Y L L P T A A A G L L L L A A Q P A M A A L Q A S R
NotI
GCGGCCGCT TATCCATACGACGTACCAGACTACGCA GGAGGT CATCACCATCATCACCAT TAG AGATCT BglII
A A A Y P Y D V P D Y A G G H H H H H * R S

```

**Fig. 3B**

# Vectors for antibody display

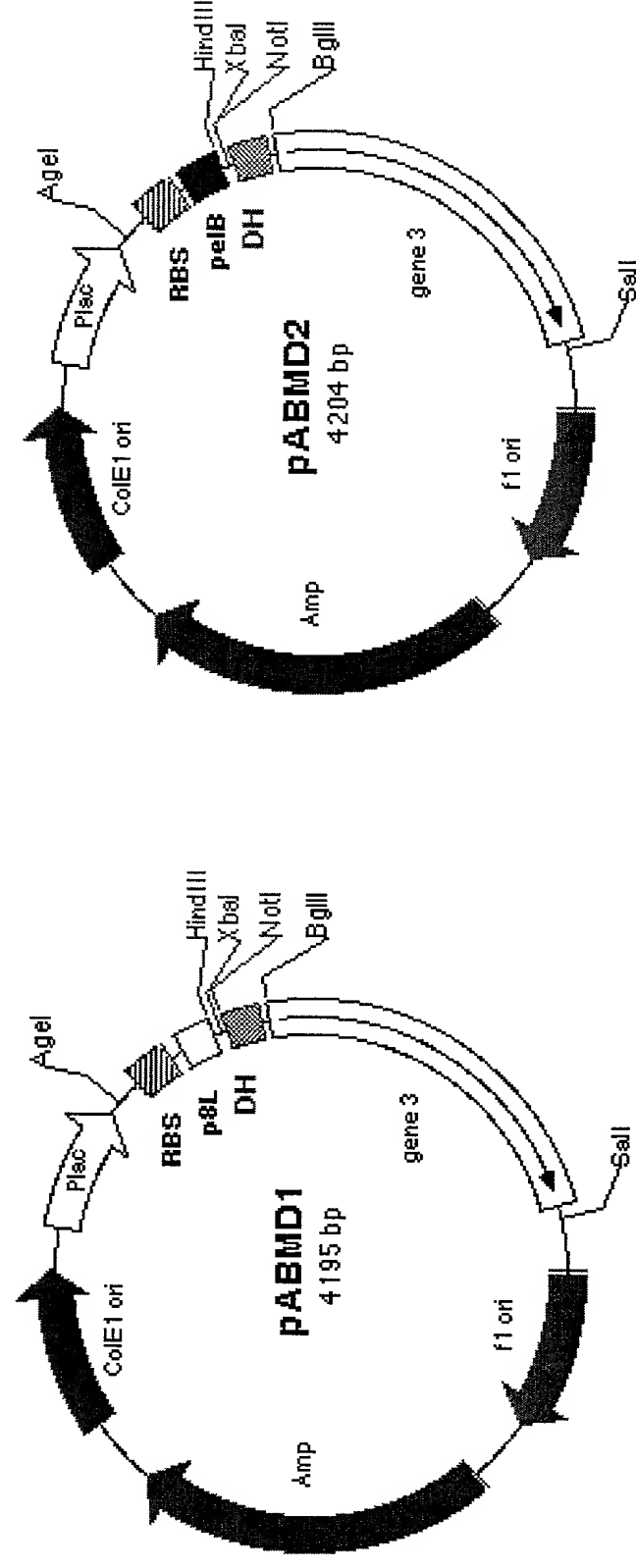


Fig. 4A

**PABMD1 vector: sequence from AgeI to Sall**

```

lac promoter/lac O1      AgeI      EP      S/D
AATTGTGAGCGGATAACAATTT ACCGGT TCTT TTAACCTTTAG TAAGGAGG AATTAAAAA
P8 Leader
ATGAAAAAGTCTTTAGTCTCAAAAGCCTCCGTAGCCGTTGCTACCCCTCGTTCGATGCTAAGCTTCGCT TCTAGA
M K K S L V L K A S V A V A T L V P M L S F A S R
NotI
GCGGCCGCT TATCCATACGACGTACCACTACGCA GGAGGT CATCACCATCATCACCAT TAG AGATCT
A A A Y P Y D V P D Y A G G H H H H H * R S
HA-tag
GGAGGCGGT ACTGTTGAAAGTTGTTTAGCAAAA ----- GCTAACATACTGCGTAATAAGGAGTCTTAA GTCGAC
G G G T V E S C L A K ----- A N I L R N K E S *
Gene 3
Sall

```

**PABMD2 vector: sequence from AgeI to Sall**

```

lac promoter/lac O1      AgeI      EP      S/D
AATTGTGAGCGGATAACAATTT ACCGGT TCTT TTAACCTTTAG TAAGGAGG AATTAAAAA
pelB Leader
ATGAAATACCTATTGCCCTACGGCAGCCGCTGGATTGTTATTACTCGCGGCCAGCCGCCCTGCAGGCCCTCTAGA
M K Y L L P T A A A G L L L L A A Q P A M A A L Q A S R
NotI
GCGGCCGCT TATCCATACGACGTACCACTACGCA GGAGGT CATCACCATCATCACCAT TAG AGATCT
A A A Y P Y D V P D Y A G G H H H H H * R S
HA-tag
GGAGGCGGT ACTGTTGAAAGTTGTTTAGCAAAA ----- GCTAACATACTGCGTAATAAGGAGTCTTAA GTCGAC
G G G T V E S C L A K ----- A N I L R N K E S *
Gene 3
Sall

```

Fig. 4B

# Vectors for ccFv expression

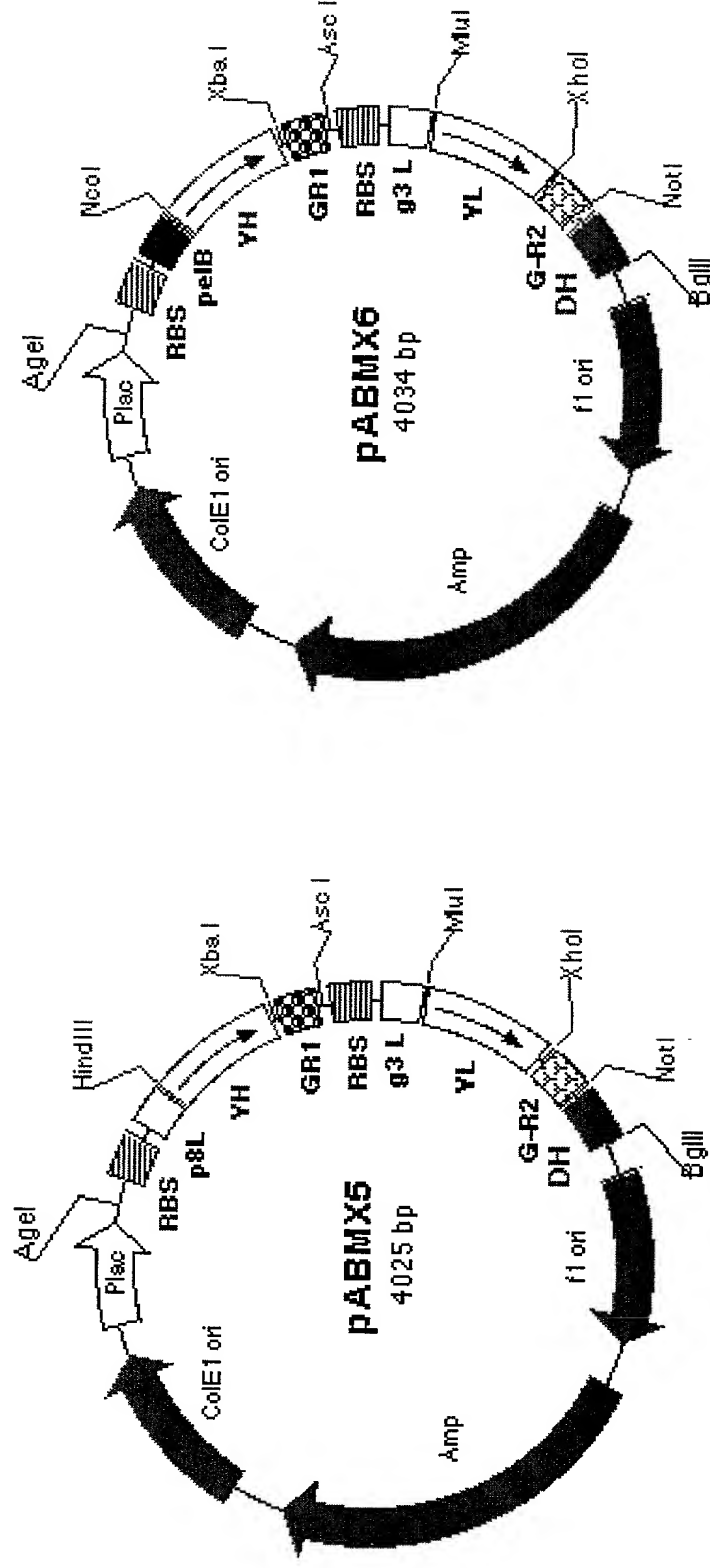


Fig. 5A

# **PABMX5 vector: sequence from p8 leader to DH-tag**

P8 Leader  
 ATGAAAAAGTCTTTAGTCTCTCAAGCCTCCGTAGCCGTTGCTACCCCTCGTTCCGATGCTAAGCTTCGCT VH XbaI  
 M K K S L V L K A S V A V A T L V P M L S F A S R  
 AscI GR1 GCGCGGCCACAATTTACAGTAAGGAGGTTTAACTT ATGAAAAAATTATTATTCGCAATTCCCTTTAGTTGTTCTCT  
 S/D VL TCTCGA GR2 GCGGCCGCTTATCCATACGACGTACGACTACGCA  
 MluI XhoI NotI HA-tag  
 TTTCTATTCTCACTCCGCTACGCGT VL TCTCGA GR2 GCGGCCGCTTATCCATACGACGTACGACTACGCA  
 F Y S H S A T R S R A A A Y P Y D V P D Y A  
 His-tag  
 GGAGGT CATCACCATCATCACCAT TAG  
 G G H H H H H H \*

# **PABMX6 vector: sequence from pelB leader to DH-tag**

pelB Leader  
 ATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCGGCCAGCCGCGCATGGCG VH XbaI  
 M K Y L L P T A A A G L L L L A A Q P A M A S R  
 Nco I  
 AscI GR1 GCGCGGCCACAATTTACAGTAAGGAGGTTTAACTT ATGAAAAAATTATTATTCGCAATTCCCTTTAGTTGTTCTCT  
 S/D VL TCTCGA GR2 GCGGCCGCTTATCCATACGACGTACGACTACGCA  
 MluI XhoI NotI HA-tag  
 TTTCTATTCTCACTCCGCTACGCGT VL TCTCGA GR2 GCGGCCGCTTATCCATACGACGTACGACTACGCA  
 F Y S H S A T R S R A A A Y P Y D V P D Y A  
 His-tag  
 GGAGGT CATCACCATCATCACCAT TAG  
 G G H H H H H H \*

Fig. 5B



# Vectors for ccFv display

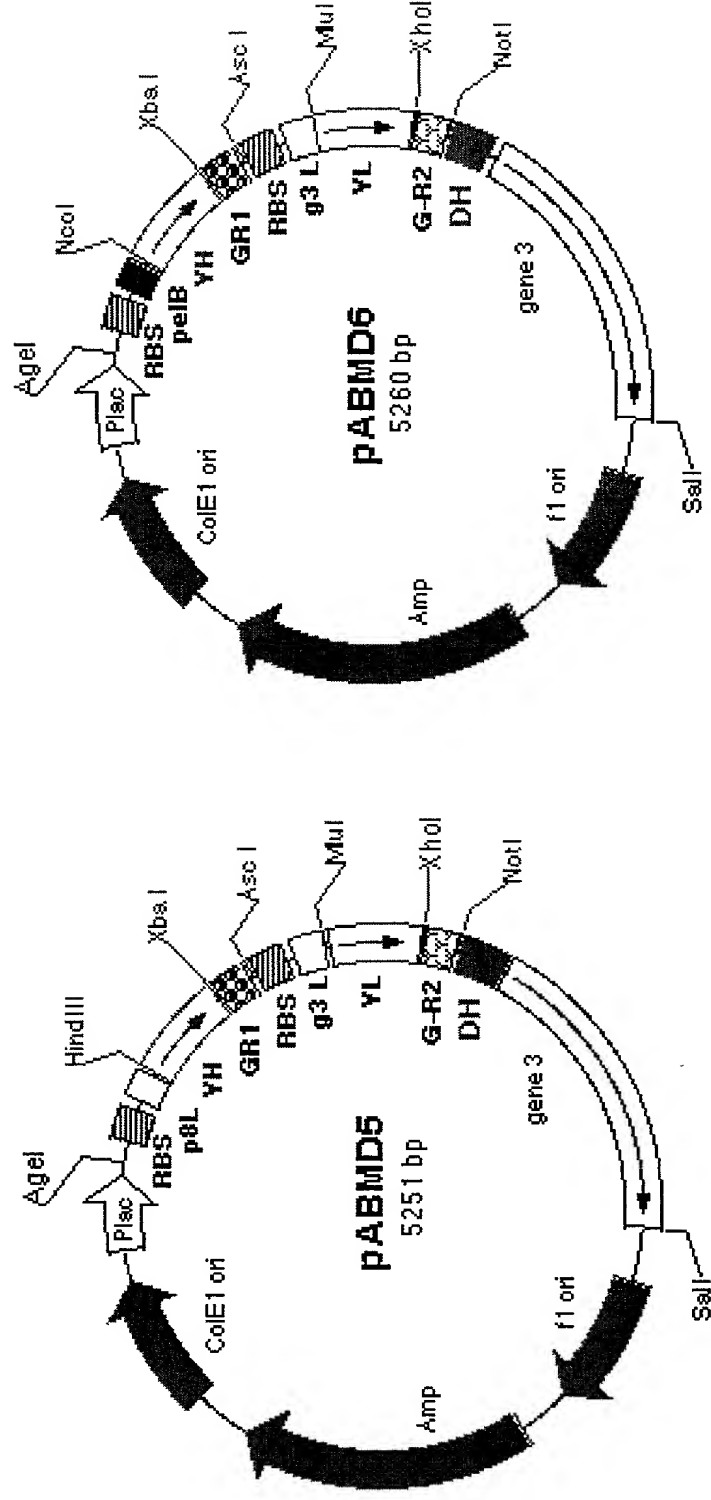


Fig. 6A

**PABMD5 vector: sequence from HindIII to SalI**

P8 Leader  
 ATGAAAAAGTCTTAGTCTCAAGCCTCCGTAGCCGTTGCTACCCCTCGTTCCGATGCTAAGCTTCGCT VH XbaI  
 M K K S L V L K A S V A T L V P M L S F A S R  
 AscI  
GR1 GCGCGCCACAATTTACAGTAAGGAGGTTAACTT ATGAAAAAATATTATTTCGCAATTCCTTTAGTTGTTCTCT  
 M K K L L F A I P L V P  
 S/D  
 P3 Leader  
 His-tag  
 MluI XhoI NotI HA-tag  
 TTCTATTCTCACTCCGCTACGCGT VL TCTCGA GR2 GCGGCCGCTTATCCATACGACGTACCAGACTACGCA  
 F Y S H S A T R S R A A Y P Y D V P D Y A  
 His-tag  
 GGAGGT CATCACCATCATCACCAT TAG GGAGGCGGT ACTGTTGAAAGTTGT---CTGCGTAATAAGGAGTCTTAA GTCGAC  
 G G H H H H H \* G G G T V E S C --- L R N K E S \*  
 SalI

**PABMX6 vector: sequence from pelB leader to DH-tag**

pelB Leader  
 ATGAAATACCTATTGCCCTACGGCAGCCGCTGGATTGTTATTACTCGCGGCCAGCCGCCATGGCG VH XbaI  
 M K Y L L P T A A A G L L L L A A Q P A M A S R  
 Nco I  
 AscI  
GR1 GCGCGCCACAATTTACAGTAAGGAGGTTTAACTT ATGAAAAAATATTATTTCGCAATTCCTTTAGTTGTTCTCT  
 M K K L L F A I P L V P  
 S/D  
 P3 Leader  
 His-tag  
 MluI XhoI NotI HA-tag  
 TTCTATTCTCACTCCGCTACGCGT VL TCTCGA GR2 GCGGCCGCTTATCCATACGACGTACCAGACTACGCA  
 F Y S H S A T R S R A A Y P Y D V P D Y A  
 His-tag  
 GGAGGT CATCACCATCATCACCAT TAG GGAGGCGGT ACTGTTGAAAGTTGT---CTGCGTAATAAGGAGTCTTAA GTCGAC  
 G G H H H H H \* G G G T V E S C --- L R N K E S \*  
 SalI

**Fig. 6B**

## Vector for ccFv expression in Yeast

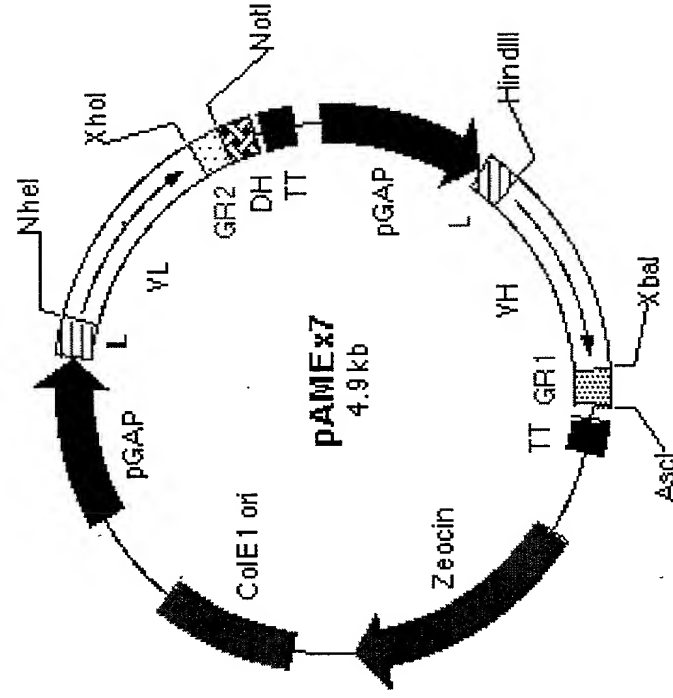


Fig. 7

## Soluble antibody ELISA

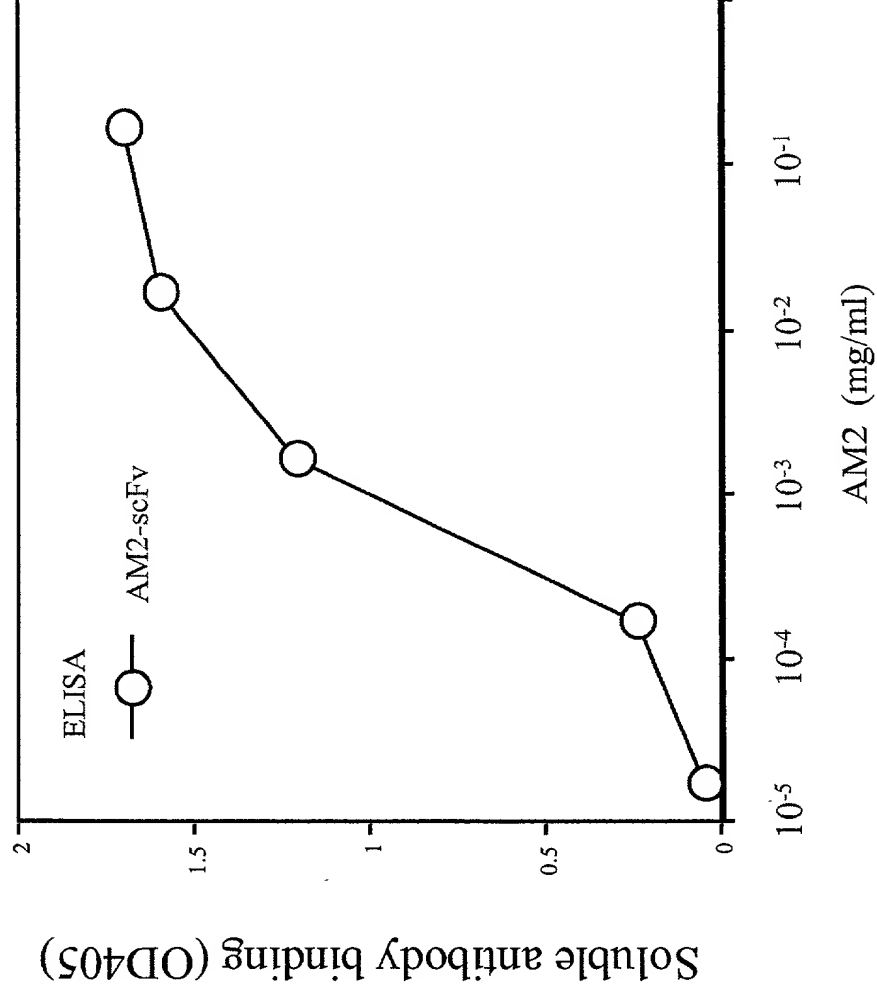


Fig. 8

# AM2-scFv display on phage by pABMD1 vector

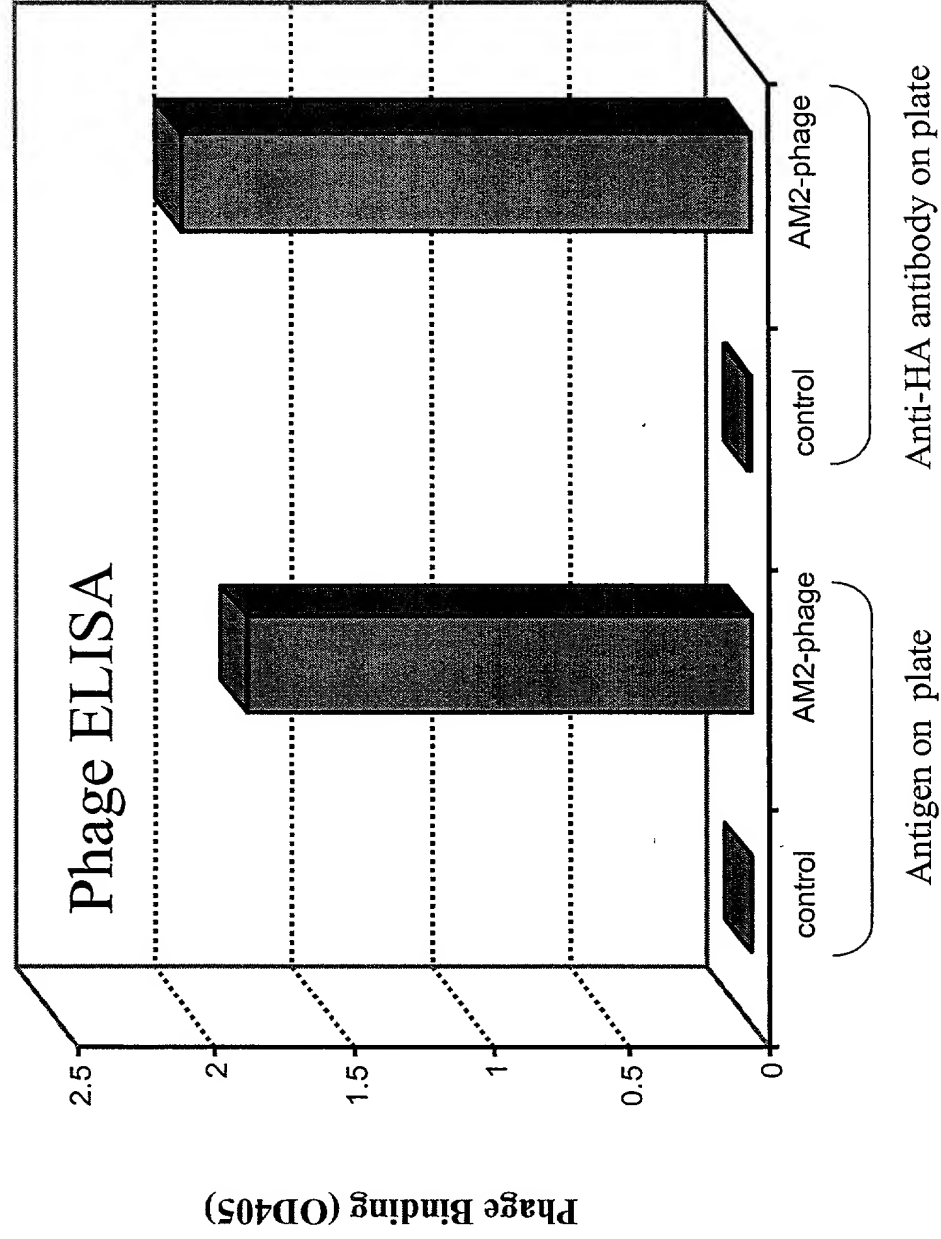


Fig. 9

# **Soluble AM1-ccFv antibody expression in *E. coli***

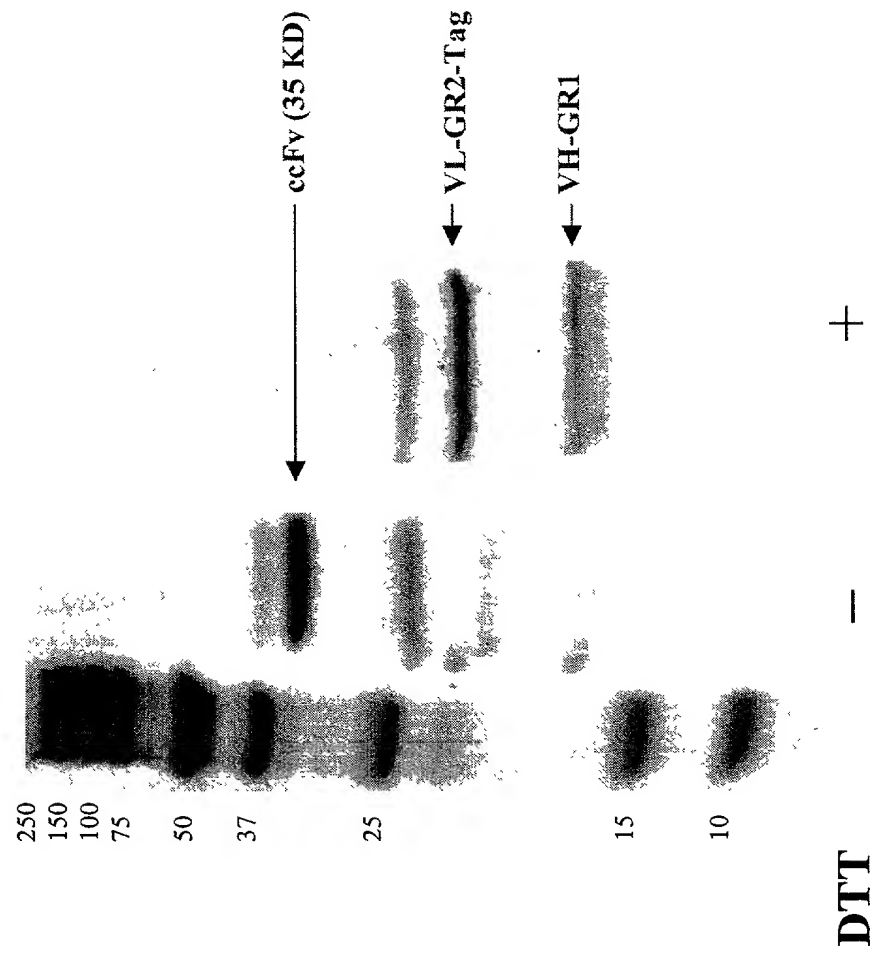


Fig. 10A

## Soluble AM1-ccFv antibody binds to antigen

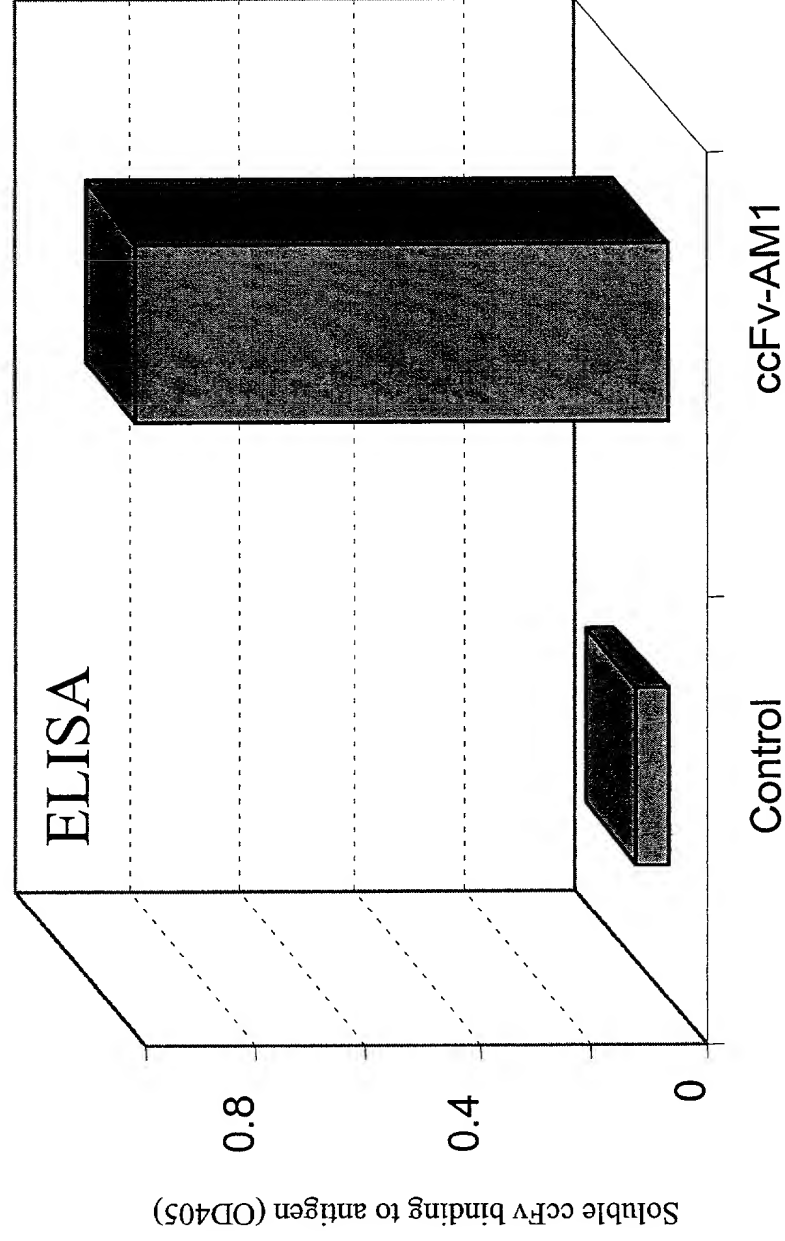


Fig. 10B

# AM1-ccFv Antibody display on phage

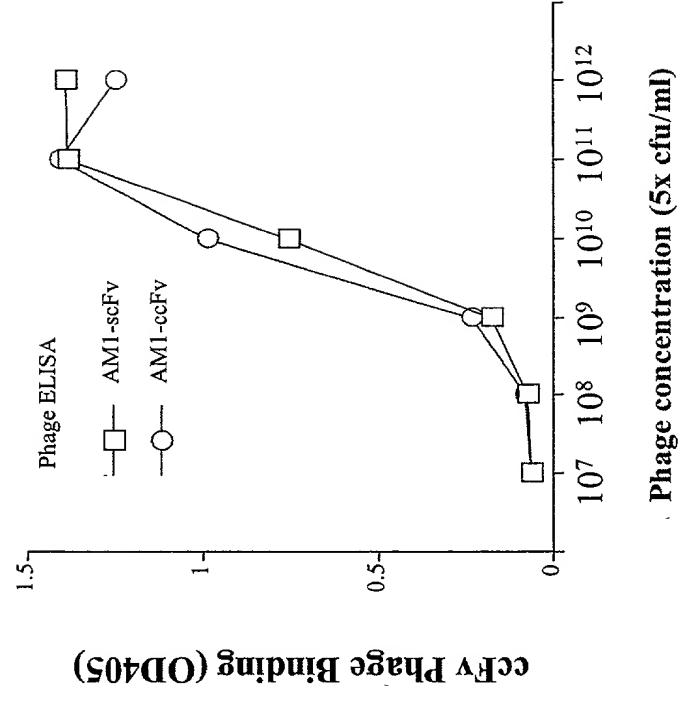


Fig. 11A



# Comparison of antigen binding capability of AM2-ccFv and AM2-scFv displayed on phage particles

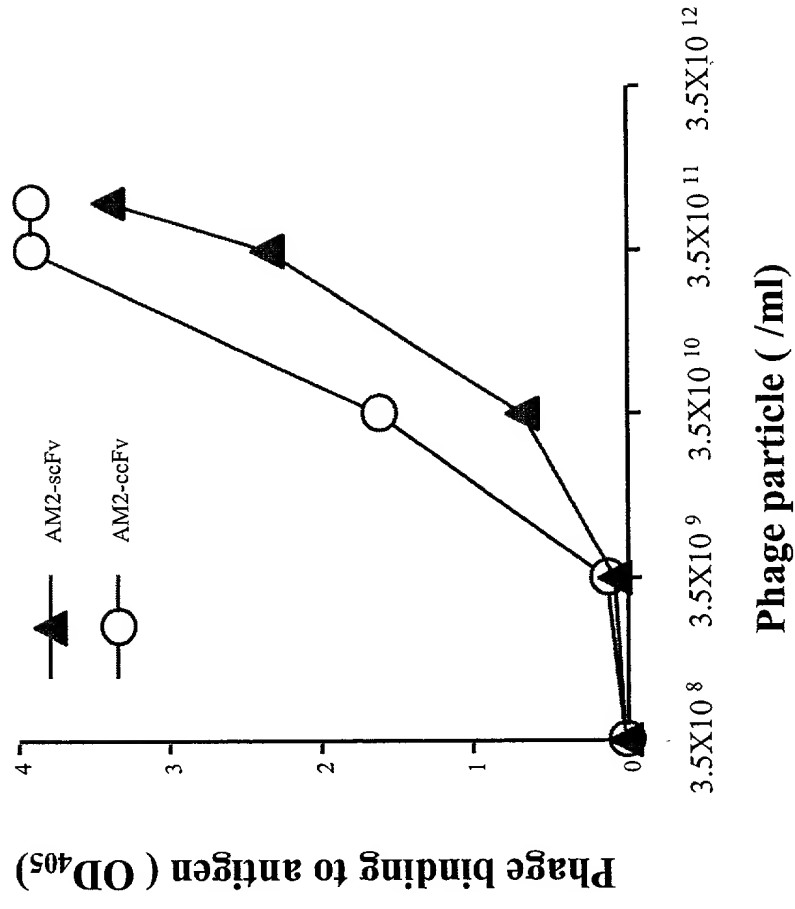
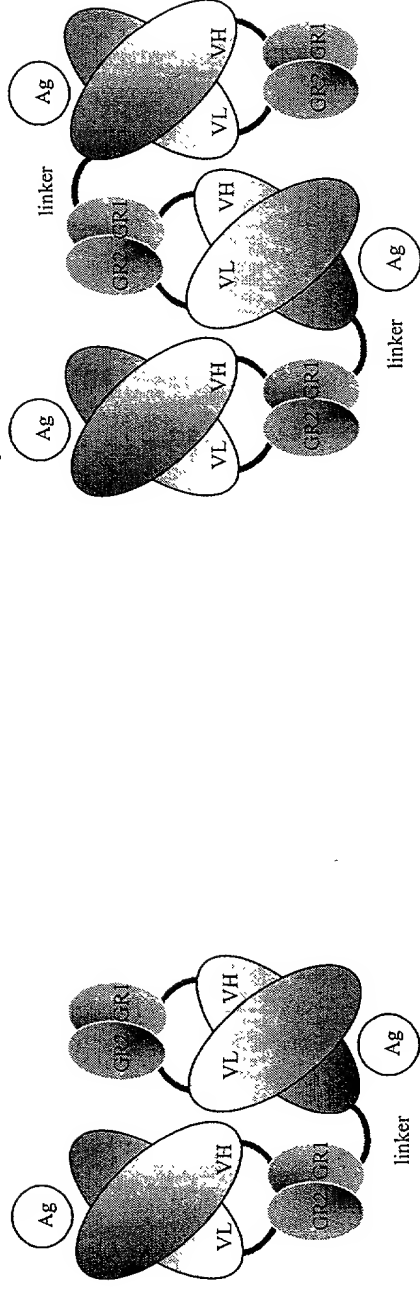


Fig. 11B

# Multi-valent ccFv antibody



trivalent (ccFv)<sub>3</sub> peptide 1: VH-GR1-VH-GR1-VH-GR1  
peptide 2: VL-GR2

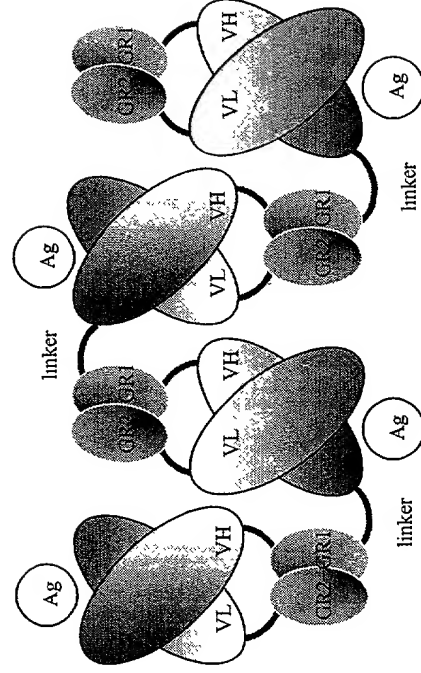


Fig. 12

# Bi-valent ccFv-scFv/dsFv antibody

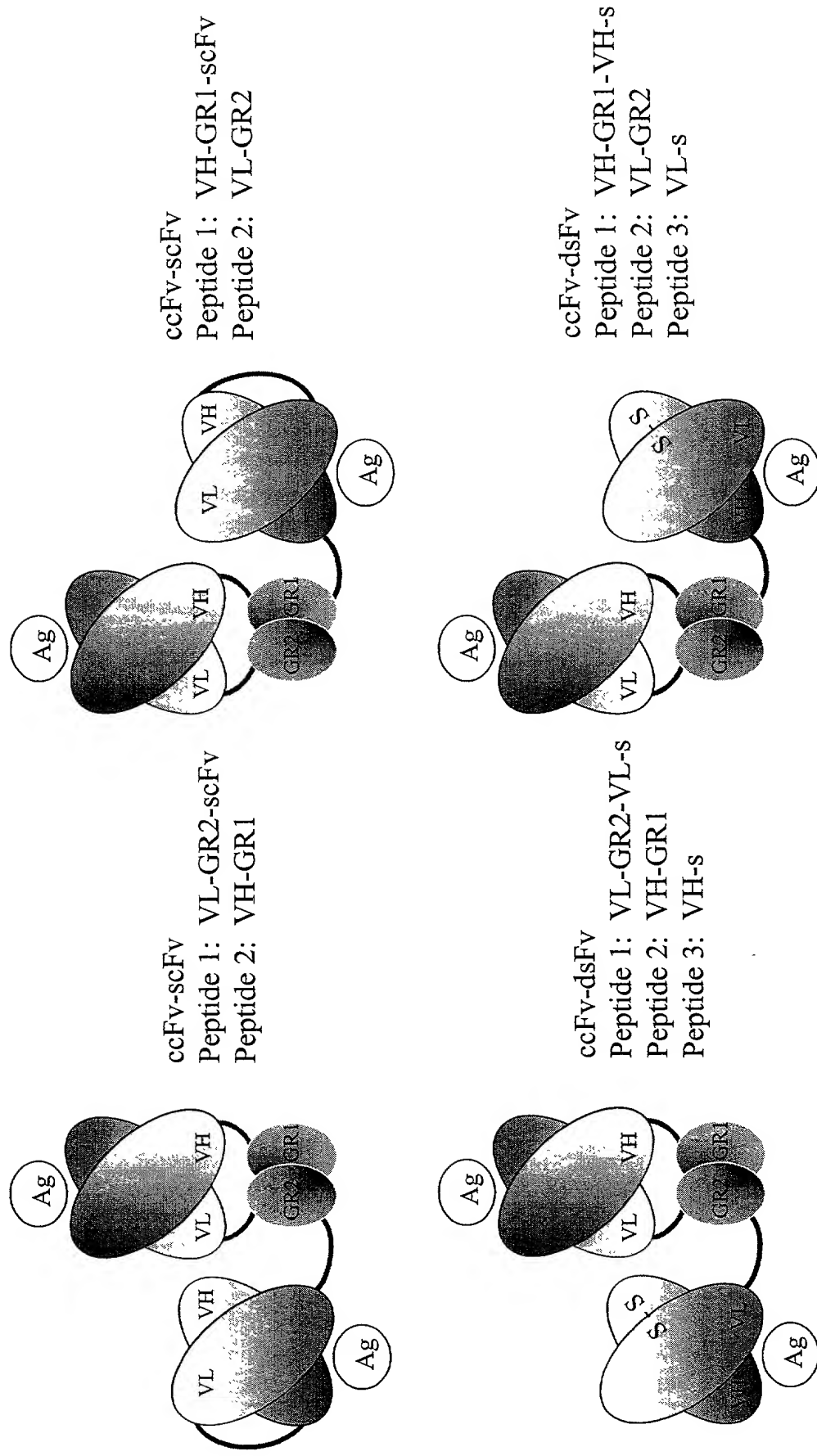
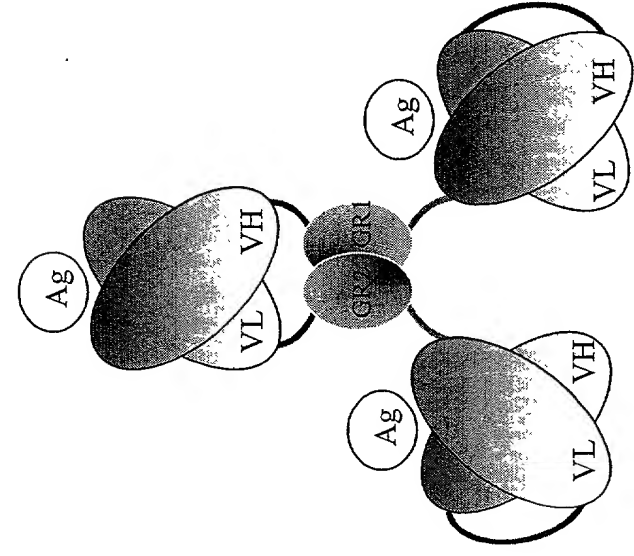
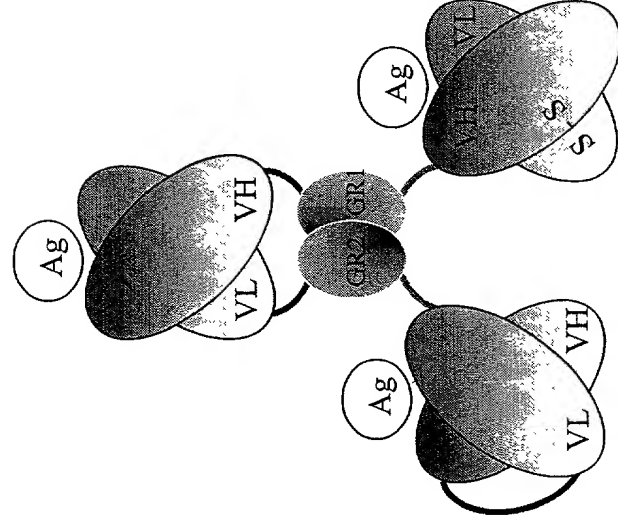


Fig. 13

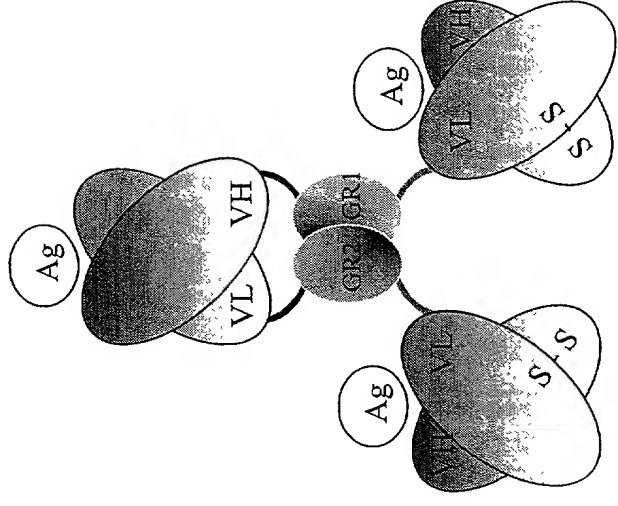
# Tri-valent ccFv-scFv/dsFv antibody



Tri-valent ccFv-(scFv)2  
 Peptide 1: VH-GR1-scFv  
 Peptide 2: VL-GR2-scFv



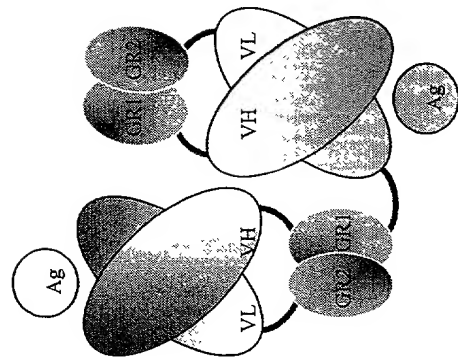
Tri-valent ccFv-scFv-dsFv  
 Peptide 1: VL-GR2-scFv  
 Peptide 2: VH-GR1-VH-s  
 peptide 3: VL-s



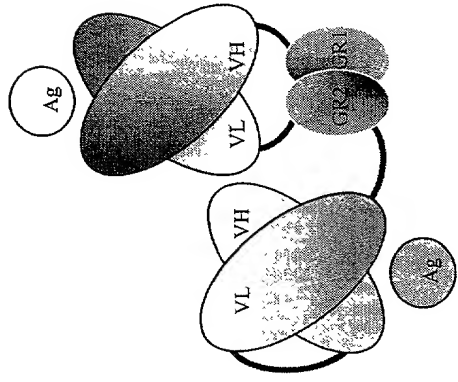
Tri-valent ccFv-(dsFv)2  
 Peptide 1: VL-GR2-VL-s  
 Peptide 2: VH-GR1-VL-s  
 peptide 3: VH-s

Fig. 14

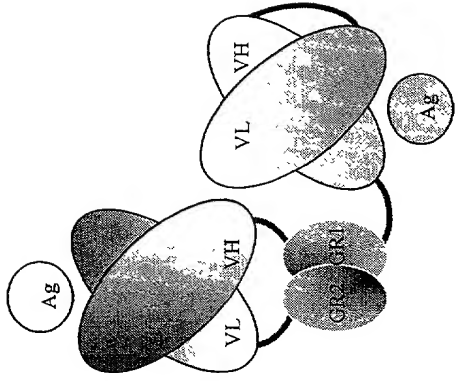
# Bi-specific antibody (1)



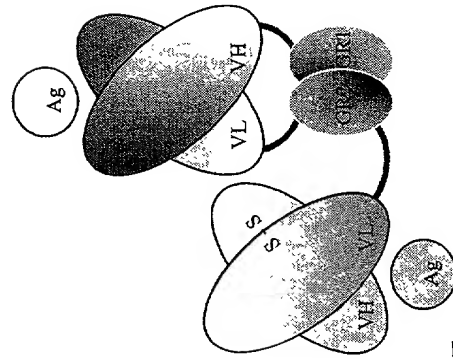
Bi-specific ccFv-ccFv'  
 Peptide 1: VH-GR1-VL'-GR2  
 Peptide 2: VL-GR2  
 Peptide 3: VH'-GR1



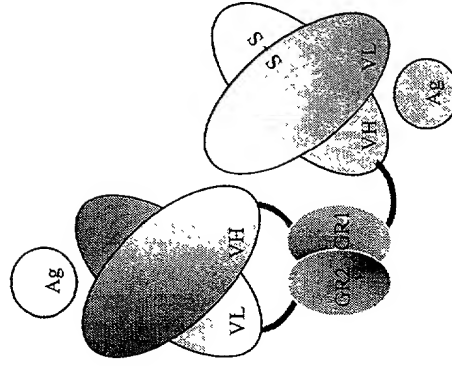
Bi-specific ccFv-scFv'  
 Peptide 1: VL-GR2-scFv'  
 Peptide 2: VH-GR1



Bi-specific ccFv-scFv'  
 Peptide 1: VH-GR1-scFv'  
 Peptide 2: VL-GR2



Bi-specific ccFv-dsFv'  
 Peptide 1: VL-GR2-VL'-s  
 Peptide 2: VH-GR1  
 Peptide 3: VH'-s



Bi-specific ccFv-dsFv'  
 Peptide 1: VH-GR1-VH'-s  
 Peptide 2: VL-GR2  
 Peptide 3: VL'-s

Fig. 15

## Bi-specific antibody (2)

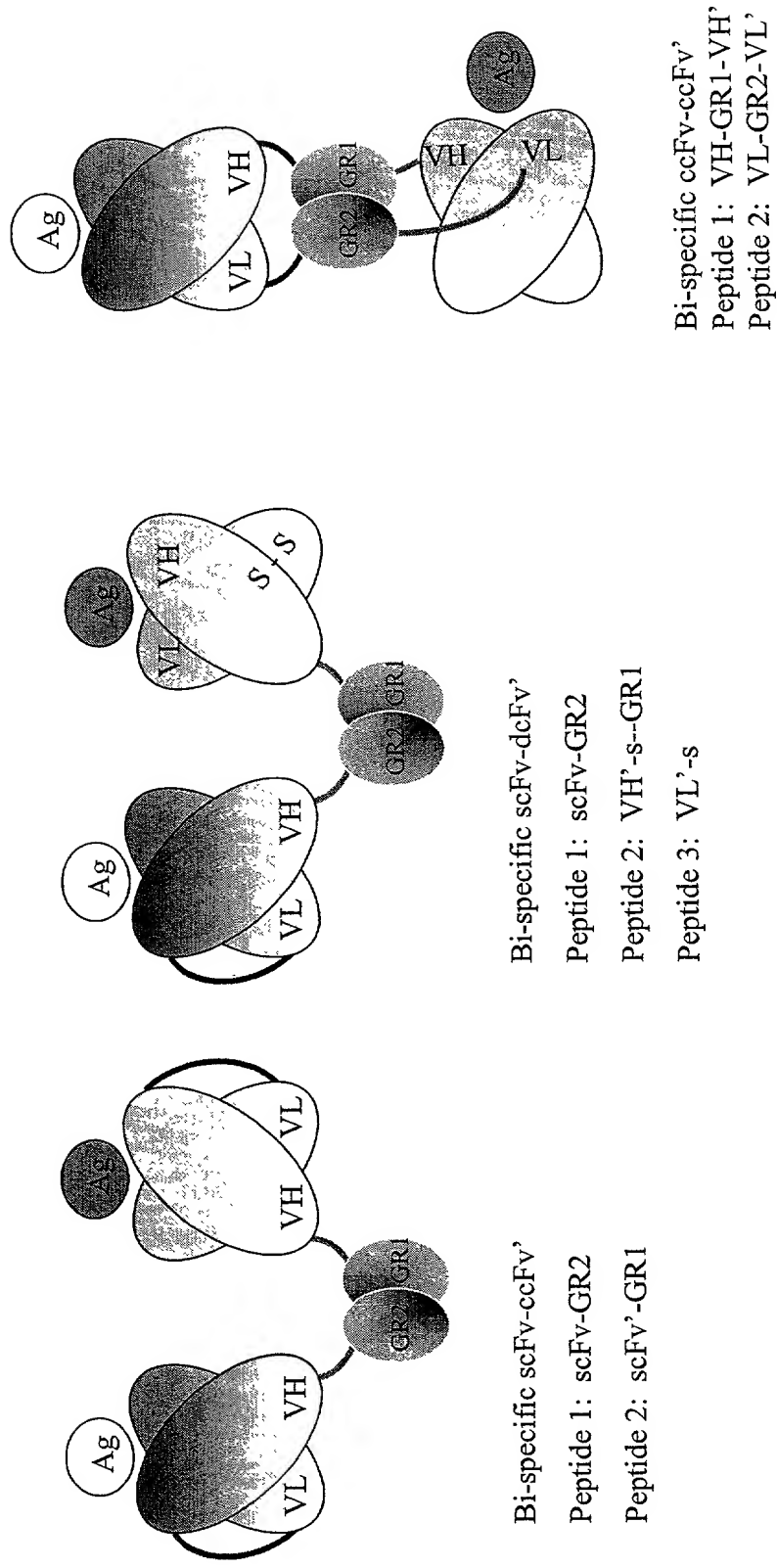
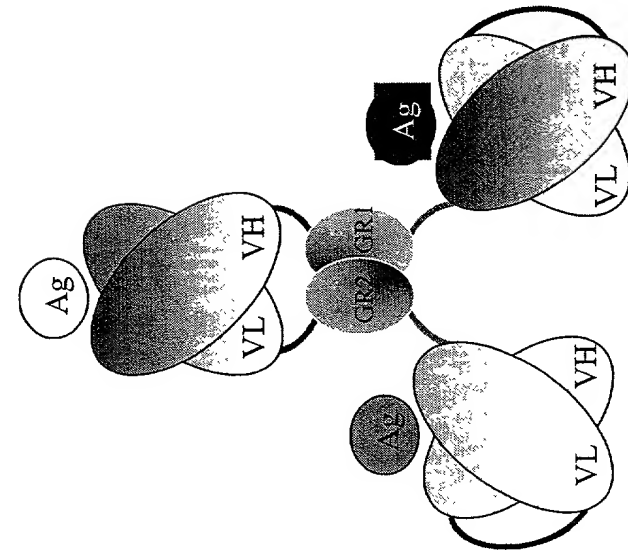
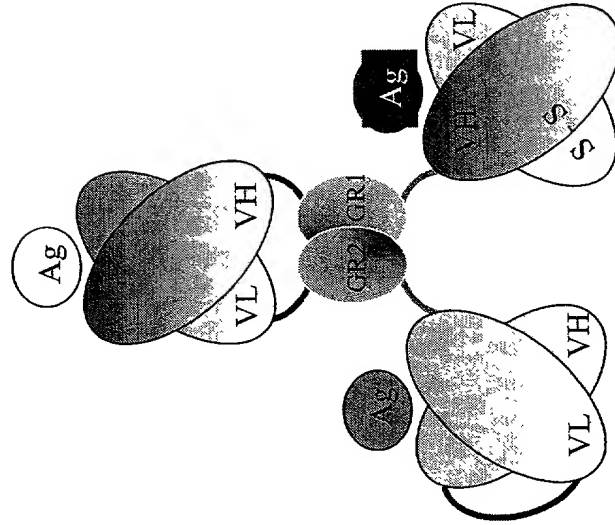


Fig. 16

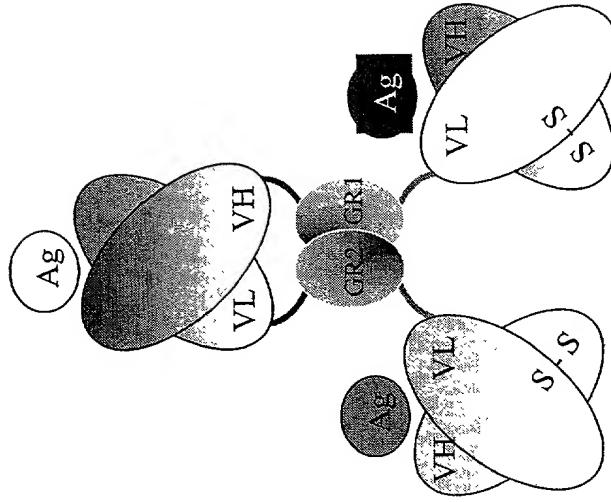
## Tri-specific antibody



Tri-specific ccFv-scFv'-scFv'\*  
 Peptide 1: VH-GR1-scFv'  
 Peptide 2: VL-GR2-scFv'\*



Tri-specific ccFv-scFv'-dsFv\*  
 Peptide 1: VL-GR2-scFv'  
 Peptide 2: VH-GR1-VH\*-s  
 peptide 3: VL\*-s



Tri-specific ccFv-dsFv'-dsFv\*  
 Peptide 1: VL-GR2-VL'-s  
 Peptide 2: VH-GR1-VL\*-s  
 peptide 3: VH'-s  
 peptide 4: VH\*-s

Fig. 17

# Single-chain ccFv antibody

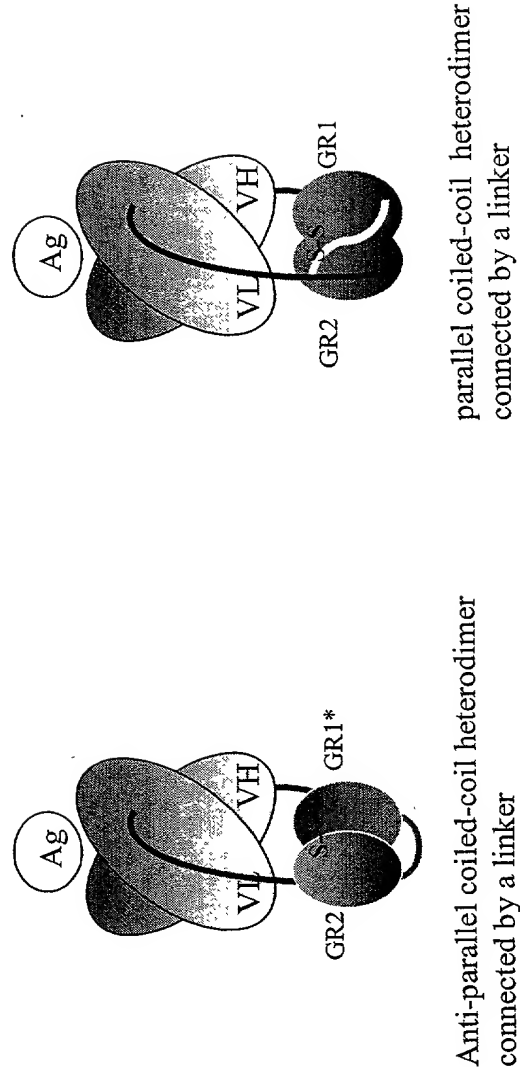


Fig. 18



## ccFv display and its use in antibody library construction

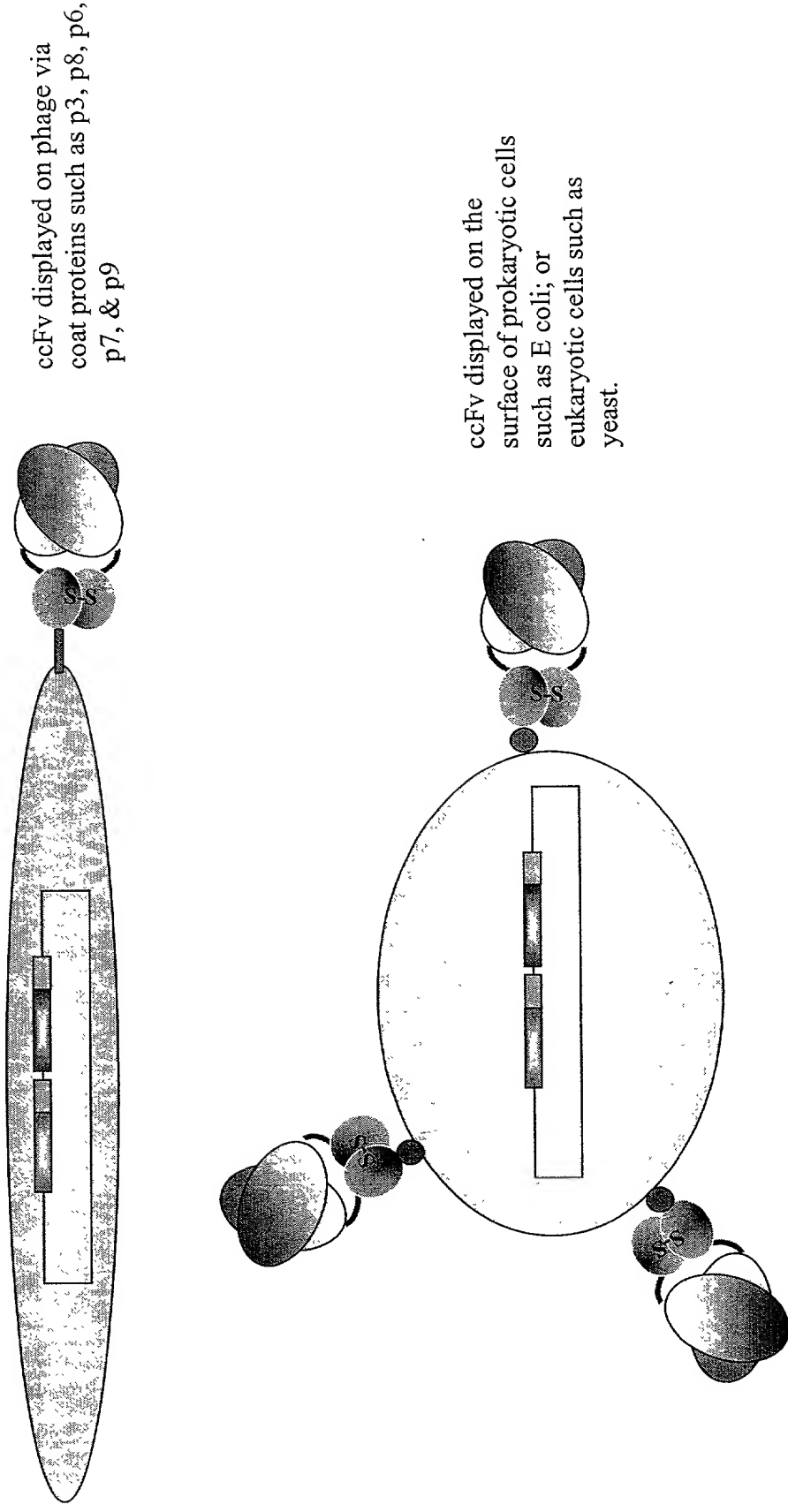


Fig. 19